DENISON UNIVERSITY BULLETIN

Volume 48, No. 2

JOURNAL

OF THE

SCIENTIFIC LABORATORIES

Volume 41 Articles 2–3 Pages 39 to 66

EDITED BY

W. A. EVERHART

Permanent Secretary Denison Scientific Association

2. Observations on the Michigan Flora: A Survey of the St. Ignace Causeway (Mackinac County). By EDWARD C. VOSS..... 39
3. The Male Frog Pregnancy Test. By CARL F. BRANDFASS, JR. AND EMIL J. MASSA.... 46

Report of the Secretary: *THE DENISON SCIENTIFIC ASSOCIATION*..... 66

GRANVILLE, OHIO

DECEMBER, 1948

The Denison University Bulletin is published bi-monthly and entered as second class mail matter at the Post Office at Granville, Ohio, under Act of August 24, 1912.

JOURNAL OF THE SCIENTIFIC LABORATORIES OF DENISON UNIVERSITY

The entire file of volumes 1 to 13 was destroyed by fire; no publications issued prior to 1907 are now available. Volumes 14 to date may be obtained from the editor at $2.00 per volume, with the exception of volume 15, the price of which is $1.00. Separate parts, as listed below, may be purchased at the prices indicated.

VOLUME 20

Articles 1-3, pp. 1-36; November, 1922.....$0.50
A review of the Biology of Sex-Determination; Sidney I. Kornhauser. 21 pp., 8 figs.
The Meander Patterns of Rios Securé and Mamoré, Eastern Bolivia; Kirtley F. Mather. 7 pp., 2 figs.
Primitive Musical Instruments of the Denison Collection; Karl H. Eschman. 10 pp., 3 plates.

Articles 4-8, pp. 37-186; June, 1923.....$0.75
Notes on Medinan, Niagaran, and Chester Fossils; Aug. F. Foerste. 84 pp., 13 plates.
The Egg and Larvae of Hesperia juba Bdv.; A. W. Lindsey. 7 pp., 1 plate.
The Occultation of Venus by the Moon on January 13, 1923; P. Biefeld. 4 pp., 1 plate.
A Botanical Survey of the Campus of Denison University; Dwight Munson Moore. 23 pp., 7 figs., 3 plates.
The Underground Migration of Oil and Gas; Kirtley F. Mather. 31 pp., 1 fig.

Articles 9-13, pp. 187-383; December, 1924.....$1.25
Trichoptilus pygmaeus Wism. and the Neuration of the Family Pterophoridae; A. W. Lindsey. 6 pp., 6 plates.
Notes on American Paleozic Cephalopods; Aug. F. Foerste. 76 pp., 22 plates.
A Report on the Theory of Relativity (Einstein Theory); Paul Biefeld. 20 pp.
Some Problems of Taxonomy; A. W. Lindsey. 18 pp., 1 plate.
Notes on the Geology of Giles County, Virginia; George D. Hubbard and Carey G. Croneis. 72 pp., 1 plate.

VOLUME 21

Articles 1-3, pp. 1-116; March, 1925.....$1.25
Notes on Cephalopod Genera; Chiefly Coiled Silurian Forms; Aug. F. Foerste. 70 pp., 23 plates.
The Cornell University Entomological Expedition South America of 1919-1920. Scientific Results No. II. Hesperiodea; A. W. Lindsey. 44 pp., 6 plates.
Photographic Record of the Partial Solar Eclipse of January 24, 1925; Paul Biefeld. 2 pp., 1 plate.

Article 4, pp. 117-283; September, 1925.....$1.50
The Agricultural Geography of the Salt Lake Oasis; Charles Langdon White. 167 pp., 34 figs.

Articles 5-7, pp. 285-404; September, 1926.....$1.25
Actinosiphonate, Trochoceroid and other Cephalopods; Aug. F. Foerste. 100 pp., 22 plates.
Brachiopods from the St. Clair Limestone, Arkansas: Norman L. Thomas. 18 pp., 1 plate.
Notes on the Preparation of Hydrogen Sulfide and Hydrogen Iodide; W. C. Ebaugh. 2 pp.

VOLUME 22

Articles 1-4, pp. 1-135, March, 1927.....$1.50
Ordovician and Silurian Cephalopods of the Hudson Bay Area; Aug. F. Foerste and T. E. Savage. 108 pp., 24 plates, 1 map.
Notes on Phylogeny in Erynnis Schr. (Thanaos Auct.); A. W. Lindsey. 7 pp., 1 fig.
The Blue Ridge of Southern Virginia and Western North Carolina; Frank J. Wright. 17 pp., 4 figs., 2 plates.
Gravels of the Blue Ridge; Frank J. Wright. 3 pp., 1 fig.

Articles 5-9, pp. 137-193, October, 1927.....$1.00
Science and Religion (Address); Robert A. Millikan. 10 pp.
Forty Years of Scientific Thought Concerning the Origin of Life (Address); Kirtley F. Mather. 11 pp.
Darwin as a Pioneer in Evolution (Address); George A. Dorsey. 14 pp.
Science and Living (Address); C. Judson Herrick. 9 pp.
Founding of the Denison Scientific Association (Address); Alfred D. Cole. 6 pp.

VOLUME 23

Articles 1-2, pp. 1-126, January, 1928.....$1.50
American Arctic and Related Cephalopods; Aug. F. Foerste. 110 pp., 29 plates.
Apatodonosaurus, A New Genus of Ichthyosaurs from the Jurassic of Wyoming; M. G. Mehl. 16 pp., 2 figs., 6 plates.

Articles 3-5, pp. 127-230, July, 1928.....$1.50
Some Brachiopods from the St. Clair Limestone, Arkansas; Norman L. Thomas. 13 pp., 1 plate.
The Phytosauria of the Wyoming Triassic; M. G. Mehl. 32 pp., 9 figs., 3 plates.
A Restudy of some of the Ordovician and Silurian Cephalopods described by Hall; Aug. F. Foerste. 58 pp., 8 plates.

Articles 6-8, pp. 231-355, December, 1928.....$1.50
Hesperioidea from the Kartabo District of British Guiana; A. W. Lindsey. 5 pp., 5 figs.
A Restudy of American Orthoconic Silurian Cephalopods; Aug. F. Foerste. 85 pp., 28 plates.
The Erosional History of the Blue Ridge; Frank J. Wright. 24 pp., 10 plates.

VOLUME 24

Articles 1-5, pp. 1-113, April, 1929.....$1.50
Hypoparia and Opisthoparia from the St. Clair Limestone, Arkansas; Norman L. Thomas. 26 pp., 1 plate.
Ordovician and Silurian of American Arctic and Subarctic Regions; Aug. F. Foerste. 54 pp., 2 plates.
Location Factors in the Iron and Steel Industry of Cleveland, Ohio; Charles Langdon White. 16 pp., 4 figs., 1 plate.
A Photographic Record of the Total Eclipse of the Moon; Paul Biefeld. 2 pp., 1 plate.
Basslerina, A New Holliniform Ostracode Genus, with Description of New Pennsylvanian Species from Texas and Oklahoma; Raymond C. Moore. 15 pp., 3 plates.

Articles 6-9, pp. 115-264, August, 1929.....$1.50
Some Proparia from the St. Clair Limestone, Arkansas; Norman L. Thomas. 14 pp., 2 plates.
Cephalopods of the Red River Formation of Southern Manitoba; Aug. F. Foerste. 107 pp., 29 plates.
A Large Fish Spine from the Pennsylvanian of North Central Texas; Raymond C. Moore. 7 pp., 1 plate.
Location Factors in the Iron and Steel Industry of the Buffalo District, New York, Charles Langdon White. 20 pp., 6 figs.

Articles 10-13, pp. 265-427, December, 1929.....$1.50
Three Studies of Cephalopods; Aug. F. Foerste. 117 pp., 23 plates.
A New Genus of Mosasaurs from Mexico, and Notes on the Pelvic Girdle of Platecarpus; M. G. Mehl. 18 pp., 5 figs., 4 plates.
Stream Piracy near Asheville, N. C.; Frank J. Wright 8 pp., 1 fig., 1 plate.
Motor Vehicle Transportation Cost and its Relation to Highway Finance; Bruce D. Greenshields. 6 pp., 1 fig.

VOLUME 25

Articles 1-3, pp. 1-164; April, 1930.....$1.50
Port Byron and Other Silurian Cephalopods; Aug. F. Foerste. 124 pp., 2 figs., 25 plates.
The Iron and Steel Industry of Youngstown, Ohio; Charles Langdon White. 22 pp., 7 figs.

OBSERVATIONS ON THE MICHIGAN FLORA: A SURVEY OF THE ST. IGNACE CAUSEWAY (MACKINAC COUNTY)

EDWARD G. VOSS[1]

One who crosses the Straits of Mackinac can hardly fail to notice a long structure like a breakwater extending from the southern shore of the upper peninsula of Michigan over a mile west of the ferry route. The United States Lake Survey concisely describes the situation: "A causeway has been constructed by the State of Michigan in a southerly direction from the south side of Graham Point about 1 mile west of its extremity; it extends about 4,000 feet from shore to the 12-foot contour."[2] (See figures 1 and 2.)

The causeway was built by the Michigan State Highway Department during investigations concerning the feasibility of constructing a bridge across the Straits of Mackinac. The causeway was completed in the spring of 1944, and this idle pile of large limestone blocks now extends almost a mile into the Straits and is approximately the width of a four-lane highway. The top is filled in with gravel and crushed stone and leveled so as to permit a car to travel the entire length. There is a short bridge near the north (shore) end of the causeway.

The State Highway Department informs me (personal communication) that the fill on the top of the causeway was all obtained from immediately north of the site, from the great excavation which provided some of the limestone blocks themselves. No grass seed was sown on the causeway. It may then be assumed that all plants currently found growing there were not brought in from elsewhere in the state during the actual construction of the mole.

On July 2 and August 20, 1948, I collected plants on the causeway in order to determine and record just what species have become established since the project was completed and abandoned in 1944. In order to give a definite limit to the collecting area, I collected only south of the bridge near the north end. With the exception of a few muddy spots where rain water accumulates, the surface of the causeway is poor soil—mostly loose gravel, sand, and disintegrating limestone.

Occasionally fishermen and curious tourists go out on the causeway, and their visits would account for the introduction of some of the plants. Another portion of the flora may be attributed to the Herring Gulls which frequent the southern end.

It is interesting to note that a greater variety of species occurs on the windward (west) side. It would seem that seeds carried by the wind and deposited as soon as they struck have germinated in spite of the difficulties of poor soil and

[1] Class of 1950, Denison University.

[2] U. S. Lake Survey, Survey of the Northern and Northwestern Lakes, Bulletin No. 57 (April, 1948), p. 128.

FIG. 1. Looking southwesterly toward the causeway from the upper peninsula mainland. From the bluff near the flagpole in Straits State Park.

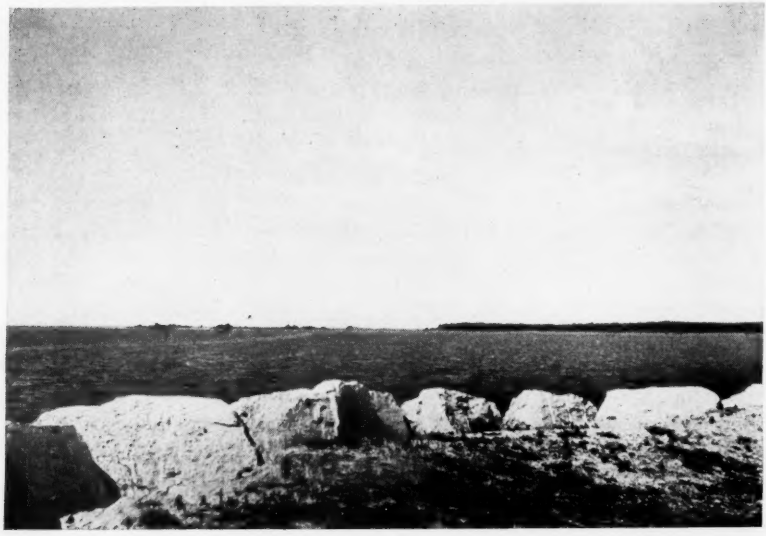

FIG. 2. On the south end of the causeway, looking west toward Green Island and Pt. La Barbe. (Both illustrations from Kodachromes by David O. Voss.)

strong winds. The stunted growth of the plants, particularly at the southern end, is obvious; measurements of a few species are given in the annotated list which follows. Good specimens for determination were sometimes scarce or

lacking. Continued exposure to winds may be expected to cause the migration of species to the leeward side (if one may speak of a "leeward" side to so narrow and exposed a situation). In low spots on the eastern side, where the soil is a little better, growths of sweet clover (*Melilotus*) and clover (*Trifolium*) three to five feet tall are comparatively dense and extensive.

In the following list of species found on the causeway, names marked with an asterisk are of species not previously reported specifically for Mackinac County in the papers listed at the end. I have attempted to keep the nomenclature of the list as up-to-date as possible; names used in Gray's *Manual* (7th ed.) are given as synonyms when different. The order of species follows the *Manual*.

I am indebted to Dr. Rogers McVaugh, curator of phanerogams at the University of Michigan Herbarium, for supplying several determinations and checking others, and to Dr. Frank C. Gates, professor of taxonomy and ecology at Kansas State College, for advice and encouragement.

SYSTEMATIC LIST OF THE VASCULAR FLORA OF THE ST. IGNACE CAUSEWAY

Poaceae:

1. *Phleum pratense* L.
2. *Agrostis alba* L.
3. *Agrostis hyemalis* (Walt.) BSP
4. *Poa compressa* L.
5. *Hordeum jubatum* L.
6. *Elymus canadensis* L.

Juncaceae:

7. *Juncus balticus* Willd. var. *littoralis* Engelm.

Iridaceae:

8. *Sisyrinchium montanum* Greene (In part *S. angustifolium*)

Salicaceae:

9. *Salix lucida* Muhl.
10. *Salix* sp.

    Some undetermined, stunted specimens, probably representing at least one or two hybrids.

11. *Populus tremuloides* Michx.

    Very small.

12. *Populus balsamifera* L.

    Young shoots rather common.

Betulaceae:

13. *Betula papyrifera* Marsh (*B. alba* var. *papyrifera*)

    Several young plants less than two dm. tall and one larger plant, somewhat deformed.

Polygonaceae:

14. *Rumex crispus* L.

Caryophyllaceae:

15. *Cerastium vulgatum* L. var. *hirsutum* Fries

    Very common. Noticed especially at southern end of causeway.

16. *Lychnis alba* Mill.
    Near southern end of the bridge.
17. *Silene cucubalus* Wibel (*S. latifolia*)
    Near southern end of the bridge.

Ranunculaceae:

18. *Ranunculus acris* L.
    Several small plants, and one tall one at the southern end of the causeway (particularly conspicuous there because other vegetation at the end is decidedly dwarfed).
19. *Aquilegia canadensis* L.
    Scarce.

Brassicaceae:

20. *Lepidium virginicum* L.
    Common. Plants only about 1 dm. tall. Noticed especially at the end.
21. *Capsella bursa-pastoris* (L.) Medic.
    Common near end, where the plants are about 4 cm. tall. Attains 25 cm. closer to shore.
22. * *Diplotaxis muralis* (L.) DC.
    Believed by McVaugh to be this species, which is not much known in Michigan. It is abundant on the half of the causeway toward the shore, and is sparingly distributed through to the southern end.
23. *Erysimum cheiranthoides* L.
    Small (5 cm. tall).

Grossulariaceae:

24. *Ribes cynosbati* L.

Rosaceae:

25. *Fragaria virginiana* Duchesne
    Common.
26. *Potentilla monspeliensis* L.
    Very common. Flowering plants are as low as 5 cm. at end of causeway.
27. *Potentilla fruticosa* L.
    Scarce.
28. *Potentilla anserina* L.
    Abundant.
29. *Rubus strigosus* Michx. (*R. idaeus* var. *aculeatissimus*)
    A plant 15 cm. tall bore 10 flowers and buds July 2! Other small plants sparsely scattered.
30. *Rosa blanda* Ait.
    A fairly good-sized shrub just south of the bridge.
31. *Rosa* sp.
    A few very small plants, not in flower, had leaves which would indicate they were escapes.

Fabaceae:

32. *Trifolium pratense* L.
    Rather common.

33. *Trifolium repens* L.
34. *Trifolium hybridum* L.
35. *Trifolium procumbens* L.
36. *Melilotus officinalis* (L.) Lam.

A few plants near the southern end of the bridge.

37. *Melilotus alba* Desv.

Very common, forming dense patches along east side.

38. *Medicago sativa* L.

Hypericaceae:

39. *Hypericum perforatum* L.

Elaeagnaceae:

40. *Shepherdia canadensis* (L.) Nutt.

Small plants rather common.

Onagraceae:

41. *Epilobium angustifolium* L.
42. *Epilobium adenocaulon* Haussk.

Several small plants with *Lobelia kalmii* and *Lycopus americanus* in low spots.

43. *Oenothera* sp.

Some specimens could probably be referred to "muricata," while the majority demonstrate the confusing array of characteristics typical of the allied and as yet unnamed species. Capsules of two of the plants have been sent to Dr. H. H. Bartlett, who is studying their progeny in his research on *Oenothera* at the University of Michigan Botanical Gardens.

Ammiaceae:

44. *Pastinaca sativa* L.

Scarce.

45. * *Daucus carota* L.

Scarce.

Cornaceae:

46. *Cornus stolonifera* Michx.

Common; some plants still in flower on August 20. This is one of the species the presence of which can probably be attributed to the fact that the gulls eat the fruit from bushes on shore and leave the indigestible seeds on the causeway.

Ericaceae:

47. *Arctostaphylos uva-ursi* (L.) Spreng. var. *coactilis* Fern. & Macb.

Scarce.

Boraginaceae:

48. *Echium vulgare* L.

One plant, covered with blue blossoms and therefore quite conspicuous. The species is abundant along many of the roadsides near St. Ignace, so its occurrence on the causeway is not surprising.

Lamiaceae:

49. *Satureja glabella* (Michx.) Briquet var. *angustifolia* (Torr.) Svenson (*S. glabra*)
    Several plants in low spots, amid grasses.
50. *Lycopus americanus* Muhl.
    In the same situations as the preceding. Rather common.

Scrophulariaceae:

51. *Verbascum thapsus* L.
52. *Chaenorrhinum minus* (L.) Lange (*Linaria minor*)
53. * *Veronica peregrina* L. var. *xalapensis* (HBK.) Pennell
    Several patches about halfway out, in mud of center strip of vegetation between divided roadway.

Plantaginaceae:

54. *Plantago rugelii* Dcne.
    Rather common.

Lobeliaceae:

55. *Lobelia kalmii* L.
    Flowering plants as low as 7 cm. in height; rather common in low places.

Compositae:

56. *Solidago altissima* L.
    Common.
57. *Solidago ohioensis* Riddell
58. *Solidago graminifolia* (L.) Salisb.
    Common.
59. *Aster lindleyanus* T. & G.
60. * *Aster laevis* L.
    "Apparently a deformed or stunted plant of this," writes McVaugh.
61. * *Aster junceus* Ait.
62. *Anaphalis margaritacea* (L.) B. & H. var. *intercedens* Hara.
    Common, especially toward the shore end.
63. *Rudbeckia serotina* Nutt. (*R. hirta* in part[3])
    Scarce.
64. *Achillea millefolium* L.
    Very common.
65. *Anthemis cotula* L.
    Common on the southern half.
66. * *Matricaria matricarioides* (Less.) Porter (*M. suaveolens*)
    Common on the northern half. The species also grows along roadsides (gravel and U. S. 2) on the mainland.
67. *Chrysanthemum leucanthemum* L. var. *pinnatifidum* Lecoq & Lamotte
    Common.
68. * *Tanacetum huronense* Nutt.
69. *Artemisia caudata* Michx.
    Rather common.

[3] Cf. *Rhodora*, 50(595): 172 ff., 1948.

70. *Senecio pauperculus* Michx. (*S. balsamitae*)
71. *Cirsium arvense* (L.) Scop.
72. * *Centaurea maculosa* Lam.
    Scarce. This species grows on the mainland along a gravel roadside in section 15, Moran Township (and doubtless along other roadsides).
73. *Taraxacum officinale* Weber
    Common.
74. *Hieracium aurantiacum* L.
    Common, especially toward the northern end.
75. * *Hieracium florentinum* All.
    Common.

LITERATURE

All species in the preceding list except the nine marked with an asterisk have previously been reported specifically for Mackinac County in one or more of the following papers.

Dodge, C. K., "The Flowering Plants, Ferns and their Allies of Mackinac Island." *Fifteenth Report of the Michigan Academy of Science* (1913), pp. 218–237, 1913. (Republished by Edwin O. Wood in *Historic Mackinac* (Vol. II), pp. 641–678, 1918.)

Dodge, C. K., "Observations on the Flowering Plants, Ferns, and Fern Allies on and near the Shore of Lake Huron from Linwood Park near Bay City, Bay County, to Mackinaw City, Cheboygan County, Including the Vicinity of St. Ignace, Mackinac and Bois Blanc Islands, Mackinac County." Michigan Geological and Biological Survey, Publication 31 (Biol. Series 6), pp. 15–74, 1921.

Potzger, J. E., "The Vegetation of Mackinac Island, Michigan: An Ecological Survey." *The American Midland Naturalist*, 25(2): 298–323, 1941.

Potzger, J. E., "The Vegetation of Round Island (Straits of Mackinac), Michigan." *Butler University Botanical Studies*, VI: 116–122, 1944.

THE MALE FROG PREGNANCY TEST*

CARL F. BRANDFASS, JR. AND EMIL J. MASSA

TABLE OF CONTENTS

I BRIEF HISTORY OF PREGNANCY TESTS 47
II THE DISCOVERY OF THE MALE FROG TEST 48
III METHODS 48
1. Care and Handling of Frogs 48
2. Urine Injected 49
3. Treatment of Toxic Urine 50
4. Technic of Injection 51
5. Microscopic Examination of Frog Urine 51
IV ENDOCRINOLOGY 55
V PHYSIOLOGY OF REACTION IN THE MALE FROG 58
VI RESULTS 58
1. Known Pregnancies 58
2. Precocity of the Reaction 59
3. Post Parturition 60
4. Comparison of Results with Friedman Tests on Suspected Pregnancies 60
5. Controls 61
6. Minimal Necessary Doses of Chorionic Gonadotropin 61
7. Speed of Reaction 61
8. Duration of Gonadotropic Activity in the Urine 61
VII CONCLUSIONS 61
1. Simplicity of Technic 62
2. Clear End Point 62
3. Speed of Reaction 62
4. Sensitivity 62
5. Specificity 62
6. Cost 62
7. Abundance of Frogs 62
VIII CLINICAL APPLICATIONS OTHER THAN ROUTINE PREGNANCY DIAGNOSIS 62
1. Diagnosis of Retained Placental Tissue 62
2. Differentiation of Threatened Abortion and Inevitable or Missed Abortion 62
IX SUMMARY 63
X BIBLIOGRAPHY 64

* The authors wish to express their grateful appreciation to Dr. W. M. Silbernagel of White Cross Hospital in Columbus, Dr. A. W. Lindsey of Denison University and Mrs. Martha S. Brandfass for their assistance in the experimental work and in the preparation of this paper. Thanks are also due to Mr. Eugene McDonald of White Cross Hospital for the Friedman tests performed on suspected pregnancies, and to Dr. J. D. Porterfield and Dr. H. R. H. Nicholas III of the Ohio Department of Health and Dr. D. R. Sperry of Newark, Ohio, for their cooperation in securing urine specimens to be tested.

All photographs are the work of Louis R. Fry, student at Denison University.

This article is the final report of a senior honors project carried on at Denison University from June, 1948, to February, 1949, in fulfillment of the special requirement for the baccalaureate degree *cum laude*. It is back dated for publication in the December, 1948, issue of this *Journal*.

BRIEF HISTORY OF PREGNANCY TESTS (11)

Several articles have appeared in the literature of the past year discussing the use of the male *Rana pipiens* frog as a new test animal for the diagnosis for pregnancy. The diagnosis of pregnancy is not a new problem of interest to the medical world, nor is the discovery of tests for its accurate diagnosis a recent accomplishment. Although not based on sound scientific knowledge, tests for diagnosis of pregnancy were performed by the ancient Egyptians over four thousand years ago.

In an article reviewing the history of pregnancy tests, Robbins writes, "It is remarkable that a condition so essentially benign and self limited as pregnancy, which must inevitably become obvious in the due course of time, has evoked so much interest in its early diagnosis." The results of recent discoveries clearly reveal an emphasis not only on the accuracy but also on the simplicity and the time required in the performance of these tests. Most of the existing tests can be classified in several categories: biological assays, chemical assays and skin tests. Numerous other tests, such as white cell counts, biopsy technics and others, cannot be specifically classified. The biological assay tests are the only existing methods which have proved reliable and accurate under rigid clinical trials. The knowledge that human pregnancy urine contains hormones capable of inducing activity in the gonads of lower animals offered the foundation upon which early pregnancy tests were founded.

The Aschheim-Zondek test, reported in 1928, marked the first major achievement in the field of pregnancy diagnosis. It remains to date one of the most widely employed of all pregnancy tests. In the original test, 1.8 to 2.4 cc of filtered morning urine were administered to immature mice in split doses over a two day period. At the end of ninety-six hours after the first injection, the animals were sacrificed and the ovaries examined for the presence of corpora hemorrhagica, a change produced by the presence of chorionic gonadotropin in the urine. Later the rat was substituted for the mouse because of greater sensitivity to the hormone and resistance to toxic factors in the urine. The technic using the rat is essentially the same except that five days are necessary to obtain a reading, and the presence of Graafian follicles or corpora lutea indicates a positive test. About two years after the first report of the Aschheim-Zondek test, a new modification was employed using the isolated female rabbit in place of the mouse or rat. This Friedman modification, although based on the same fundamental principles, has gained large popularity because of the reduction in the time element involved from five to two days (5).

In 1933 a new pregnancy test utilizing for the first time an amphibian, the female South African clawed frog, *Xenopus laevis*, was demonstrated before the Royal Society of South Africa by Shapiro and Zwarenstein. This test demonstrated the fact that amphibians react to mammalian hormones, although the difficulty of securing the test animals is a serious obstacle to its widespread adoption. This observation opened an entirely new field in pregnancy diagnosis.

THE DISCOVERY OF THE MALE FROG TEST

In 1947, Robbins and Parker reported that the male South African clawed frog, *Xenopus laevis*, would react to gonadotropic hormone by the emission of sperm (11). This observation suggested the use of this animal in a new and revolutionary pregnancy test. Although this animal later proved unsatisfactory, a new test involving the same principles was reported that same year by Mainini using the male toad, *Bufo arenarum* Hensel (6). These observations demonstrated a major modification in the fundamental principles of pregnancy testing, namely, the emmission of sperm by a male animal as an end point in place of the reactions in the ovary of the female. This test offered one great advantage due to the male animal's exhibiting a much greater sensitivity to chorionic gonadotropin and thus requiring a smaller quantity of pregnancy urine to produce a positive reaction. However, the unavailability of these toads in many areas imposes one objection to its widespread use and has led to further investigations with available amphibia.

Wiltberger and Miller reported in February, 1948, the use of the male frog, *Rana pipiens*, as a new test animal for early pregnancy (16). In this article and a later publication in May, 1948, they reported this test was accurate only during the first trimester of pregnancy (8). In a later article by Robbins and Parker reporting on this same test, no mention was made of its reliability being restricted to early pregnancy (12). They reported the results of 122 urines tested, having obtained 77 correct positive results, 44 correct negatives, and only 1 false negative.

It is the purpose of this paper to report on a series of urines we have tested using the male frog, *Rana pipiens*, as a test animal and to evaluate its reliability in the light of these results. These tests are also intended as experimental trials of previously reported observations by other workers in its use for routine pregnancy and other clinical applications. We feel that in the technic described below, some improvements or refinements in procedure have been advanced which appear to increase the accuracy and simplicity of this test.

METHODS

Care and Handling of Frogs. Male frogs measuring $3\frac{1}{2}$–4 inches body length and weighing more than 40 grams were used. The frogs were stored in a refrigerator tray and kept at a temperature of about 10°C until ready for use. In this artificial hibernation, metabolic activities were greatly reduced, and it was not necessary to feed the frogs. To prevent excessive drying out due to loss of moisture, the container was filled with water to a depth of about $\frac{3}{4}$ inch. Frogs should be wet at all times, but if placed in deep water from which they cannot emerge, they will drown in a short time. A container measuring 1 foot by 2 feet and 6 inches in depth will hold as many as 6 or 7 dozen frogs. It should be covered with mesh wire and the lid so arranged that a hinged cover at each end can be raised without removing the whole top of the container. The water in the container should be changed daily. When it is necessary to keep only a few frogs on supply, a large jar with a perforated lid will prove sufficient. The

bottom of the jar should be covered with a shallow layer of water which is changed daily.

Before using a frog it should be examined for the proper size specifications stated above. Experience will soon enable the technician to determine this at a glance. The technician should also be certain that a male frog is being used. The male *Rana pipiens* can easily be distinguished by a heavily pigmented callus on the base of the first digit (thumb).

If a frog has been used in a negative test, it may be used again as soon as desired. However, it is best to isolate the frog in a separate container for at least 24 hours and then reexamine as described below before using it again or returning it to the original container with the negative frogs, since it is possible that urines with an extremely low hormone titer may result in tests which appear to be negative after 2 hours and turn weakly positive several hours later.

If a frog is used in a positive test it should be isolated from the unused or negative frogs for at least a week and then can be used again if desired. Some reports have stated that it is not necessary to use frogs for a second injection since their low cost permits the discard of used animals (12). In this laboratory frogs have been used for more than one test, and it has had no apparent effect on the accuracy of subsequent results.

Each frog should be examined before injection for the presence of spermatozoa in the urine. Picking up a frog with the head and shoulders in the hand will cause it to eject a jet of urine. The microscopic examination of the urine will be described in this paper.

The normal breeding season may have some effect on the presence of spermatozoa in freshly collected animals. The tests described below were performed from July, 1948, to February, 1949, and reports on tests conducted in other laboratories cover the remainder of the year (16, 12, 8). In no case has sexual activity been noted. It is believed that the artificial hibernation due to the low temperature of the refrigerator and isolation from females will suppress the normal discharge of spermatozoa.

Urine Injected. When obtaining urine to be tested it is best to secure a first morning specimen. No special caution regarding diet or medication prior to passing of specimen is necessary. 25 cc of urine are sufficient for several tests and should be passed in a clean but not necessarily sterile bottle.

It is unnecessary to add any preservative to the urine as the hormone will retain its potency at room temperature for several weeks. However, the specimen should be guarded against excess heat. As soon as convenient the bottle should be placed in a refrigerator until the test is performed.

Filtration of the urine is necessary only when the sediment interferes with the injection by clogging the hypodermic needle.

The pH of the urine should be determined. Nitrazine paper with a pH range from 4.5 to 7.5 was used in this laboratory. It was discovered early in our work that most of the toxic urines which killed the frogs had a pH over 7.0. Even though the urine was made slightly acid with .1N HCl the urine was still toxic. Conversely, if a nontoxic urine was made strongly alkaline with .1N NaOH, no

toxicity was observed. Therefore, it was assumed that the alkalinity of the urine was not the cause of the toxicity, but the pH can be used as a means of detecting probable toxic specimens. For this reason the following procedure was put into practice.

Treatment of Toxic Urines. The volume of urine normally injected into each frog was 5 cc. However, if the pH was determined to be over 7.0, the urine was diluted 3:2, that is, 3 cc of urine to 2 cc of distilled water. If this injection killed the frogs, a greater dilution was tried with a slightly smaller volume of liquid injected. This usually consisted of a 1:1 dilution; 2 cc of urine to 2 cc distilled water. By carefully observing the frog and forcing him to urinate before death, a test can usually be obtained. Before dying the frogs experience irregular respiration and muscular spasms. The frog then loses all coordination in its appendages and finally straightens stiffly before dying. Many positive tests have been obtained on frogs which lived only 45 minutes. If the test is negative no diagnosis can be made unless the frog lives at least 1½ hours. If the above dilutions have been injected into several frogs and none have lived to give a test, another specimen should be obtained from the patient. If this is impossible several other procedures are available. A split dosage consisting of 1 cc injections given at half hour intervals until a total of 4 cc has been administered may enable the frog to survive the toxicity.

One method of overcoming toxicity consists of a chemical procedure for removing a concentrate of the gonadotropic hormone from the urine (9). One writer advocates the technic for all urines used in this test. We can see no advantage in using this procedure except when working with toxic urines that can be injected in no other way. It requires a larger volume (45 cc) of urine for the procedure and adds to the complexity and time required in the performance of the test.

This procedure is as follows (9):

Procedure for the Concentration of the Gonadotropic Hormone in Urine

1. Place 2 cc of brom-cresol purple indicator in a 50 cc cylinder.
2. Add urine to 45 cc mark (if less, dilute with water).
3. Shake (invert to mix dye and urine).
4. Add 5 cc of 20% HCl and mix (color is yellow). pH should be 3.0. If not, add more HCl.
5. Add 5 cc of 20% suspension of kaolin and mix.
6. Shake on Kahn Shaker in 100 cc bottles and return to original 50 cc cylinder. In an acid medium the hormone molecules adhere to the kaolin particles.
7. Let stand for one hour or until all precipitation settles to the 5 cc mark on the cylinder.
8. Decant the supernatant liquid and save the sediment.
9. Centrifuge the remaining suspension for 5 minutes so that all liquids can be decanted leaving kaolin and suspended hormone.
10. To this solid mass add 2 cc of .1N NaOH or until color becomes blue. pH should be about 8 or 9. This separates the hormone molecules and the kaolin.

11. Transfer to small tube and centrifuge for 15 to 20 minutes.
12. Decant and save the liquid. This liquid contains the concentrated hormone.
13. Add 2 cc of 5% HCl until solution turns yellow (pH 5 or 6) to nitrazine paper.
14. The remaining liquid containing the concentrated hormone is ready for injection. So that enough liquid will be injected to force the frog to urinate for a test, the concentrate should be diluted to 5 cc with water.

Death of frogs from injections of toxic urines before a test could be completed offered a problem at the beginning of our work. By the methods described above, this obstacle has been greatly reduced. Although there is a possibility of a false negative result if dilutions lower the hormone titer below the sensitivity of the frog, the accuracy of our results has not been affected where these procedures have been employed.

Technic of Injection. A hypodermic syringe equipped with a $\frac{3}{4}$ inch 25 gauge needle is filled with 5 cc of the urine to be tested. A needle of larger gauge enables the urine to leak from the frog after the needle has been withdrawn.

The skin of the frog is not closely applied to the body wall, the space between being designated as the lymph space. It is divided into 3 sections by longitudinal dorso-lateral connective tissue septa. These septa are marked externally by a ridge running from the head to the caudal region on each side (Fig. 1).

Separate injections are made into each lateral lymph sac. It was discovered early in our work that direct injection into either lymph sac resulted in leakage of the urine if the frog exercised violently. This can be a factor in false negative results if the volume of urine retained is too small to cause a reaction.

For this reason injection is made through the dorsal lymph sac into the lateral sacs. The connective tissue septum between the sacs is self-sealing and will prevent the urine from leaving the lateral sacs.

The needle is inserted through the skin of the dorsal wall mesial to the ridge separating the lymph sacs. Particular caution should be taken not to inject into or through the body wall as intraperitoneal injection may cause injury to the frog. When the point of the needle has entered the dorsal sac, the needle is swung laterally and passed through the septum into the lateral sac. Half of the urine is injected with the needle in this position, and the remaining half is injected in the same manner on the opposite side.

When making the injection the frog can best be controlled if his legs are straightened and grasped close to their proximal end (Fig. 2).

It is recommended that at least 2 frogs be injected for each test due to the possibility of varying individual sensitivity, especially when the hormone titer is low.

After injection the frog should be isolated in a clean, dry, wide-mouthed jar at room temperature. After a period of at least 2 hours has elapsed an observation can be made for a positive or negative test.

Microscopic Examination of Frog Urine. At the end of two hours any urine that has been voided by the frog is examined microscopically. If none is present

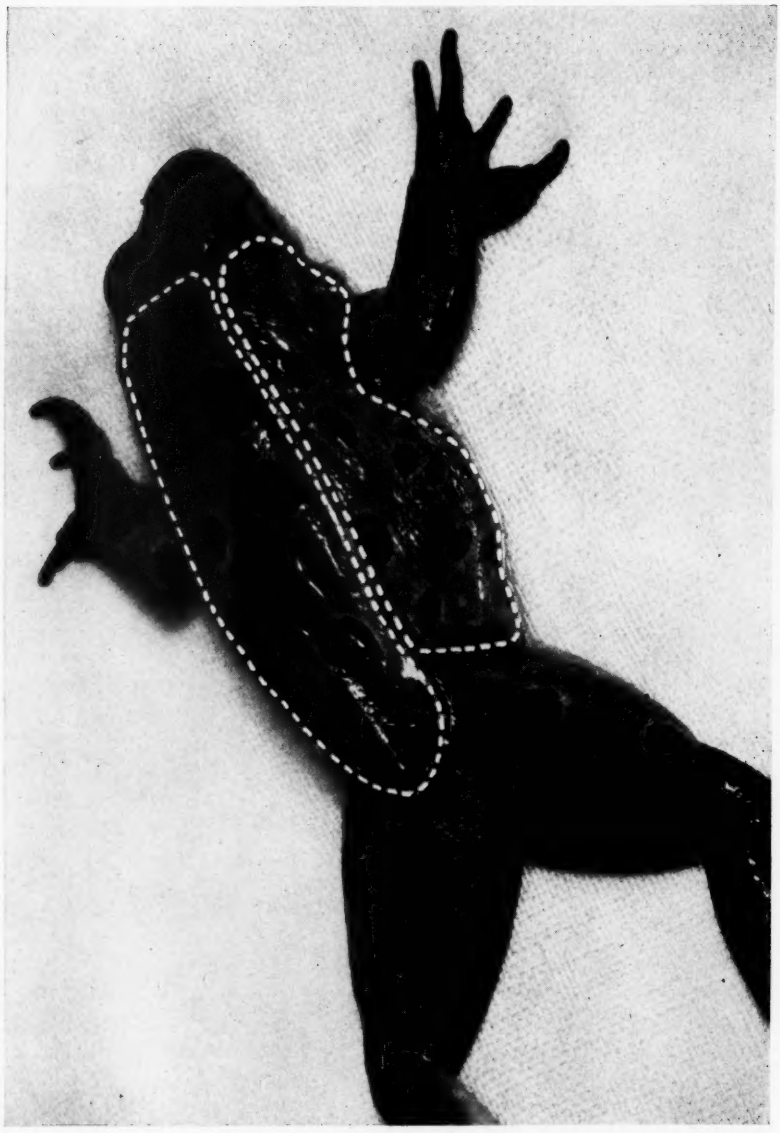

FIG. 1. Photograph of frog showing dorsal and lateral lymph sacs in outline

or if it is negative, the jar should be drained and the frog forced to urinate by seizing his head and shoulders in the hand. The urine is removed from the jar with a pipette and placed on a clean glass slide. The slide is examined under

FIG. 2. Technic of injection

a 16 mm objective for the presence of frog sperm which will be seen as small rod shaped bodies, usually exhibiting slight motility (Fig. 3). However, their presence is indicative of a positive test regardless of motility. The number of

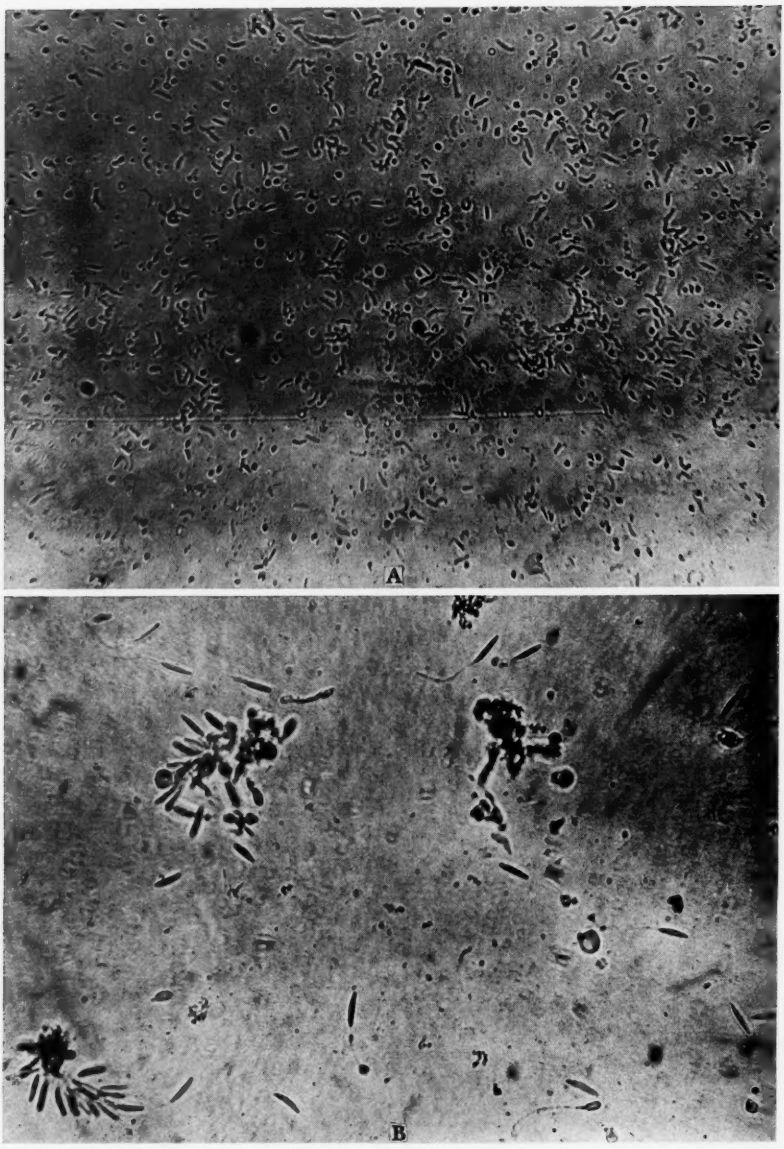

FIG. 3. A. Spermatozoa as seen in a strong positive test under 16 mm. objective
B. Spermatozoa as seen under the 4 mm. objective

sperm to be seen in the field may vary from one to many depending upon the strength of the reaction. One spermatozoon alone is indicative of a positive reaction.

It is easy at first to confuse many similar structures in the urine even though they may not be sperm. For this reason the slide should also be examined under a 4 mm objective. Under this magnification the sperm will appear as elongated cigar-shaped bodies, with parallel sides and tapering at each end (Fig. 3). Slender, refractive tails can usually be seen at one end under high power magnification with careful focus. Occasionally tails can be seen under the low power objective. The outline of the spermatozoon appears very regular as compared with other structures which may at first glance resemble spermatozoa. However, it is only in rare cases that a test is so weak that it is not instantly determined to be positive or negative.

If the test is negative at the end of 2 hours, the frog can be rechecked several hours later to confirm the results. Although this is not necessary as a part of the normal procedure, it may help to remove the very small error in percentage that is caused by a rare specimen of abnormally low hormone titer.

In most tests the spermatozoa are either abundant or the field is clear, and any subjective interpretation is eliminated.

ENDOCRINOLOGY

From all available evidence the placenta not only serves as an organ of interchange between the mother and foetus, but also functions as an endocrine gland secreting hormones whose detection at high levels of concentration in the blood or urine is the foundation of modern biological pregnancy tests. These hormones are capable of inducing activity in the gonads of lower animals, the nature of the reaction varying according to the kind and sex of the animal used and the specific hormones to which that animal is sensitive.

In our tests we have demonstrated that the male frog will react to chorionic gonadotropin derived from pregnancy urine. Although we have made no injections of other hormones, extensive investigations have been carried out by other workers on the male toad, which seems to be similar in its reaction and sensitivity to chorionic gonadotropin (6, 7). Mainini observed no response to the injection of different doses of estradiol benzoate, progesterone, testosterone propionate, desoxycorticosterone, "pitressin" N.N.R., thyroxin, adrenalin and prolactin. He also reports positive results obtained with chorionic, serous and pituitary gonadotropins, with a follicle-stimulating hormone preparation from bovine pituitary gland and with crude suspension or implantation of pituitaries from the rat, rabbit and batrachia (7).

The gonadotropic hormones have been classified in two major groups. The first group includes the chorionic gonadotropins, which are secreted only in the presence of a fetal placenta or are found in the blood and urine of either sex suffering from certain types of malignant tumors. Gonadotropins of the second group are secreted by the anterior hypophysis and can be extracted from hypophyseal tissue (15).

It has been demonstrated that the gonadotropins secreted by the placenta are similar but not identical to those secreted by the pituitary. It is believed that the gonadotropic hormones consist of two separate hormones, or one hormone acting in two different ways. Some work has been performed on the purification

and fractionating of the pituitary gonadotropins (15). One of these fractions has been called the follicle-stimulating hormone (FSH). It promotes the growth and ripening of ovarian follicles and affects the germinal epithelium of the testis. The second fraction, called the luteinizing hormone (LH) or interstitial cell stimulating hormone (ICSH), promotes the formation of corpora lutea and acts on the interstitial cells of the testis. Some workers have claimed a third fraction called luteotropin about which little is known (15). Chorionic gonadotropin secreted by the placenta appears to consist of these same fractions as evidenced by their similar physiological effects (1).

It is believed that the placenta gradually supplements the normal ovarian and pituitary functions during pregnancy by the secretion of gonadotropins, estrogens, and progesterone (4, 15). Convincing evidence exists that these hormones are secreted by the chorionic constituent of the placenta. Large amounts of gonadotropins have been extracted directly from placental tissue where they are secreted by the cells covering the chorionic villi (15). Although the gonadotropins disappear shortly after parturition, their production continues if placental fragments are retained (10). Patients suffering from chorionepithelioma, a tumor of embryonic origin, contain high levels of gonadotropins in the blood and urine (18). A hydatidiform mole in which the chorionic villi degenerate into watery sacs also secretes gonadotropic hormones (18). Further evidence that these sources secrete gonadotropins rather than merely store them has been demonstrated by growth of placental fragments, chorionic tissue from ectopic pregnancies, and hydatidiform moles in tissue cultures and were shown to secrete gonadotropins for a period of two to six months (15).

The appearance of chorionic gonadotropins in the blood, and sometimes in the urine, correlates approximately with the time of implantation of the blastocyst (15). The earliest intra-uterine blastocysts that have been observed have been eight days old and may or may not have been implanted (4). The process of implantation is begun by the blastocyst from which the extra embryonic trophoblast grows and invades the endometrium of the uterus. Cells from the developing blastocyst and uterine decidua contribute to the formation of the placenta. It has been demonstrated that the placental hormones are most abundant in the fetal part, the chorionic trophoblast (4).

The quantity of chorionic gonadotropin secreted during pregnancy varies widely in individual cases. By the time the first menstrual period is missed, gonadotropin secretion is well under way. The time at which its presence can be detected in the urine may vary. Among the cases reported for the first time in this article are one in which a positive frog test was obtained 5 days before the first missed period and several which gave positive tests within a week after the first missed period. From the time of its first appearance the level of gonadotropic hormone rises rapidly, reaching a peak at about the thirtieth day after the first missed menses. This peak may vary from 75,000 to 1,000,000 rat units per day. After the maximum has been reached the level drops rapidly until about the end of the first trimester of pregnancy when it is excreted in amounts varying from 3,000 to 10,000 rat units daily. This level is maintained, with

minor fluctuations, throughout the remaining pregnancy. Following parturition the hormone soon disappears from the blood and urine, normally being absent by the fifth day (2), (Fig. 4).

Work done in this laboratory has demonstrated that a mature male frog will react to approximately 30 international units of chorionic gonadotropin (Antuitrin "S"). No work has been reported to explain whether this reaction is caused by the follicle-stimulating or luteinizing principle. Robbins states that it is believed to be the luteinizing principle in view of the fact that follicle-stimulating hormone derived from menopausal urine has caused no reaction (11). Work performed by Zondek, Sulman and Black has suggested that only the presence of large amounts of the luteinizing hormone indicates pregnancy and

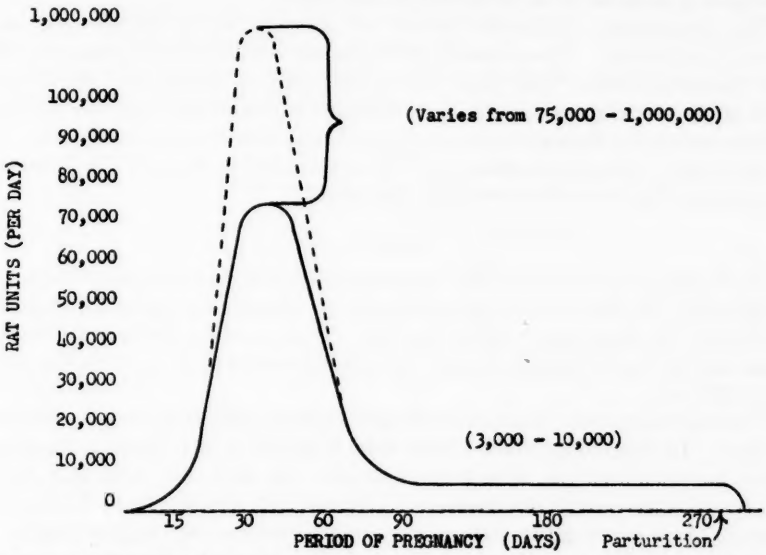

FIG. 4. Diagrammatic scheme of the daily excretion of chorionic gonadotropin during pregnancy.

that increased amounts of the follicle-stimulating hormone may be excreted in the urine in conditions other than pregnancy (polyhormonal amenorrhea, climax, tumor) (17).

Mainini has demonstrated that the male toad, *Bufo arenarum* Hensel, will react to 40 international units of chorionic gonadotropin. He has also shown that at the peak of hormonal production, 4 toads reacted to .125 cc of urine which indicated a titer of approximately 320,000 international units per liter. Urines injected from $3\frac{1}{2}$ to 6 month pregnancies gave positive tests with .5 cc of urine which indicates at least 80 international units per cc, well above the quantity required to produce a positive reaction in the male frog even if 1 cc is used for an injection (7).

In a chorionepithelioma or hydatidiform mole larger amounts of hormone are excreted, and quantitative determination by serial dilutions may be necessary to distinguish them from a normal pregnancy (18). Mainini reports well over 320,000 international units in one case of chorionepithelioma (7).

PHYSIOLOGY OF REACTION IN THE MALE FROG (6, 3)

The testicles of the frog are located in the dorsal region of the coelomic cavity and are connected to the kidney by means of a short ligament. Their general structure is similar to those of other animals, consisting of seminiferous tubules, interstitial cells and supporting tissue. Collecting tubules pass from the seminiferous tubules through the ligament into the kidney. Thence the route of excretion is common to the spermatozoa and urine.

The spermatozoa within the testicle are attached to the Sertoli cells and grouped in bundles. The gonadotropic hormones stimulate the interstitial cells and the Sertoli cells. This causes the spermatozoa to detach from the Sertoli cells in the seminiferous ampules, pass through the seminiferous tubules into the kidney and thence through the ureter to the cloaca where they are finally excreted in the urine. This phenomenon has been investigated in detail by De Robertis, Burgos and Breyter in *Bufo arenarum* Hensel (6).

RESULTS

In this laboratory a total of 211 tests have been performed to date: 104 known pregnancies, 30 post parturition specimens, 61 suspected pregnancies (parallel tests with Friedman tests), and 16 controls. Tests were also performed to determine the minimal necessary doses of chorionic gonadotropin to which the male frog will react.

Known Pregnancies. 104 tests were performed on urine from known pregnant women. In those cases where visible signs of gestation were lacking, the diagnosis was confirmed by subsequent histories. In these 104 cases of verified pregnancies the results obtained are as follows: 102 positive tests, 2 negative tests. These two negative tests must be interpreted as false negative results.

In the above false negative results, we immediately obtained another specimen from each patient. These tests gave positive results. According to Robbins, the false negative result can sometimes be explained in terms of a transitional level of the hormone titer. At the time when the urine specimen was obtained the hormonal level may have been in a rising or falling state. A second specimen obtained a few days later may reveal the newly established level (11). It is our belief that if this condition does exist, its incidence is negligible. The accuracy of our results and those reported by other workers appear to verify this assumption (12).

According to Miller and Wiltberger, 50% false results were obtained on women beyond the first trimester of pregnancy. In view of the above data this observation has not been verified as evidenced by a 98.08% accuracy throughout all months of pregnancy (16, 8).

By reference to Table 1, it is evident that these tests have been performed on patients in almost every week of pregnancy.

As further proof of the reliability of the test, Robbins and Parker have reported an accuracy of 98.72% in 78 cases of verified pregnancy, one false negative test having been obtained. No mention was made of the duration of the pregnancies from which these urines were obtained (12).

Precocity of the Reaction. Of considerable importance in the evaluation of any pregnancy test is its ability to diagnose a pregnancy in its very early stages. This appears to be one of the significant advantages of the male frog pregnancy test. Evidence in support of this statement can be found from the following case histories.

Case 1. Mrs. R. C.

Last menstrual period—October 23, 1948.
Probable date of conception—November 8.
Frog test positive on November 28.

In this case a positive result was obtained approximately 20 days after conception or 8 days after the first missed period.

TABLE 1

Results in different periods of pregnancy

	PERIOD OF PREGNANCY																																							
	Months																																								
	1				2				3				4				5				6				7				8				9									
	Weeks																																								
	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4				
Number of correct positive results	1	1	1	2	1	2	4	5	5	6	5	4	3	5	5	11	4	2	1	1	1	3	1	1	2	1	2	1	1	0	1	1	0	2	2	0				
Number of false negative results					2																																			

Case 2. Mrs. R. D.

Last menstrual period—October 21, 1948.
Probable date of conception—November 4.
Patient experienced uterine bleeding and cramping on November 23.
Frog test positive on November 28.

In this case a positive result was obtained approximately 24 days after conception or 9 days after the first missed period.

It is possible in both of these cases that a positive result may have been obtained earlier if the test had been performed. However, most women are not suspected to be pregnant until about one week has elapsed after the first missed period. At that time the frog appears capable of indicating the presence of the chorionic gonadotropins.

One outstanding case has been called to our attention (14). In this case the date of conception was accurately established by means of an isolated contact, coincident with the time of ovulation as determined by a body temperature chart. The patient soon experienced morning nausea and tenderness of breasts,

symptoms which had never been present during normal menstruation. A frog pregnancy test was performed 8 days after conception and a positive result obtained. Thus the pregnancy was diagnosed 5 days before the first missed period. It has been reported that the earliest intra-uterine blastocysts have been observed 8 days after conception, and that the presence of chorionic gonadotropin in the blood or urine is approximately coincident with implantation of the blastocyst. This case appears to verify these observations. The other 2 cases reported also reveal the advantages of this test in diagnosing early pregnancies.

Post Parturition Specimens. A limited series of tests were performed to determine at what time a test becomes negative after the termination of a pregnancy. The results of these tests are shown in Table 2. No positive tests were obtained beyond 24 hours. 2 cases remained positive for 24 hours, 4 remained positive for 12 hours, and 3 were negative within 12 hours. This is considerably sooner than the Aschheim-Zondek or Friedman tests become negative after delivery.

TABLE 2

Table showing results on 10 cases after parturition

CASES	HOURS AFTER PARTURITION				
	12	24	36	48	72
1	—	Pos.	—	Neg.	Neg.
2	—	Pos.	—	Neg.	Neg.
3	Neg.	—	—	—	—
4	Neg.	—	—	—	—
5	Neg.	—	—	—	—
6	Pos.	No more specimens obtainable.			
7	Pos.	No more specimens obtainable.			
8	Pos.	No more specimens obtainable.			
9	Pos.	Neg.	Neg.	—	—
10	Pos.	Neg.	—	—	—

In one series of tests, specimens were obtained from a patient at regular intervals following an abortion during the first trimester of pregnancy. The test was still positive after 44 hours.

At the time of a normal delivery the hormone titer is at a low level and will be excreted from the system in a short time. If an abortion takes place during the first trimester of pregnancy, the hormone titer is at its highest level and may require a longer period of time to be excreted.

Comparison of Results with Friedman Tests on Suspected Pregnancies. In order to determine the reliability of this test a series of urines from suspected pregnancies were tested and the results compared with Friedman tests performed in another laboratory. In this series of 61 tests, results were comparable in only 59 cases, one urine killing the frog and another killing both frog and rabbit. In the 59 remaining tests coincident results were obtained on 57. The frog and rabbit agreed on positive results in 31 tests and on negative results in 26. The results obtained with the frog and rabbit differed in 2 cases. In one of these

cases the frog gave a negative test and the rabbit was positive. In the other case the frog was positive and the rabbit negative. It was impossible to verify the results in either of these cases and a conclusion could not be reached. It would be erroneous to conclude that these were false results in either the rabbit or the frog since a verification was not obtainable. However, it is significant that the rabbit and frog agreed in 96.67% of the tests compared. Robbins and Parker report on 34 urines obtained from women with amenorrhea determined not to be pregnant by the Aschheim-Zondek test or its Friedman modification, and no false results were encountered in the frog test (12).

Controls. A series of control specimens were injected to demonstrate that negative results will be consistently obtained in the absence of a functional placenta or certain types of malignant tumors (chorionepithelioma and hydatidiform mole). 5 specimens from men after puberty and 11 specimens from normal menstruating women gave negative results. Mainini reports negative results in "623 control tests performed on the male toad with urine obtained from nonpregnant women, men and children, who were in normal health or with a variety of pathologic conditions. The same results were attained by others with a total of 543 control specimens of urine" (7). We consider these controls applicable to a discussion of this test because of the similar sensitivity in the toad and the frog, and in view of the fact that the gonadotropic hormones are the activating agent in both animals. Robbins and Parker further report 10 control tests on the male frog with urine secured from normal menstruating women in which only negative results were obtained. We feel it is significant that a false positive test has never been encountered.

Minimal Necessary Doses of Chorionic Gonadotropin. A series of tests were performed to determine the sensitivity of the frog to chorionic gonadotropin. In these tests we found the minimal necessary dose to be 30 international units. 10 frogs were injected with 30 units and all gave positive reactions. Frogs injected with 20 units gave no reaction.

Speed of the Reaction. In 159 positive tests performed in this laboratory the results were obtained on 158 within 2 hours after injection. In the one remaining test, the positive reaction was not evident at the end of 2 hours, but it was detected when a check was made 24 hours later. It is possible that this test might have been positive had it been checked 3 hours after injection. In numerous cases we have found the reaction time to be as short as 45 minutes to 1 hour.

Duration of Gonadotropic Activity in the Urine. In this laboratory urine specimens have been stored at refrigerator temperatures and a positive test obtained after one month has elapsed. It is an established fact that the gonadotropic activity is reduced with time, but with the injection of 5 cc of urine this factor is not considered to be a great source of error if the specimen is injected within a reasonable period of time.

CONCLUSIONS

The value of the male frog test in the routine diagnosis of pregnancy has the following advantages when compared to the present established procedures:

Simplicity of Technic. The performance of this test offers many advantages:

A. No special preparation of urine is necessary.
B. Injection is made without difficulty.
C. The collection of the frog's urine is simple and no surgical examination is required. The microscopic examination of the frog's urine for spermatozoa is readily made and requires no histologic preparation.

Clear End Point. The positive result is clearly evidenced by the presence of spermatozoa in the frog's urine. The absence of sperm clearly indicates a negative test, and the natural emission of sperm has not been encountered. The spermatozoa are easily identified because of their well-defined characteristics, and any subjective interpretation is eliminated.

Speed of the Reaction. In most cases the results can be obtained in 2 hours. We have encountered urines with high hormone titers in which a positive result was evident in 45–60 minutes. This is considerably quicker than the time required in the Aschheim-Zondek test or its Friedman modification.

Sensitivity. We have found that the frog will react to 30 international units of chorionic gonadotropin. This is well below the titer in the urine during normal pregnancy. The frog appears to be more sensitive than animals employed in other standard pregnancy tests.

Specificity. In this laboratory positive results have been obtained only with urine from pregnant women and with chorionic gonadotropin (Antuitrin "S"). Other authors have reported positive results with the urine of women having chorionepithelioma and hydatidiform mole (7). In 211 tests performed in this laboratory a false positive result has not been encountered.

Cost. In comparison with the routine tests now being employed, the expense of this test is considerably less. The animals cost in the vicinity of 15 cents, and 1 or 2 frogs suffice for each test.

Abundance of Frogs. Frogs are abundant in most areas of the United States and can be obtained during any season from biological supply houses.

CLINICAL APPLICATIONS OTHER THAN ROUTINE PREGNANCY DIAGNOSIS

Diagnosis of Retained Placental Tissue. Miller, Wiltberger and Pickett report the use of the male frog test in the diagnosis of retained placental tissue (10). They observed cases in which the test remained positive after expulsion of the foetus. Upon further examination they found live placental tissue still attached to the uterine wall. Upon curettage the test became negative within a short time.

From this observation they concluded the following: "The pregnancy test on the male frog, *Rana pipiens*, is positive as long as there is live placental tissue still adherent to the uterine wall."

Differentiation of Threatened and Inevitable or Missed Abortion. Silbernagel, Patterson and Pickett report the use of the male frog test in the differentiation of threatened abortion and inevitable or missed abortion (13). Their observations are based on the fact that a positive test implies the presence of a functioning placenta, and a negative test is interpreted as evidence that the placenta, though present, is not attached. If the patient experiences cramps and uterine

bleeding and the test is positive, she is diagnosed as threatening abortion. If these symptoms are exhibited and the test is negative, it is diagnosed as an inevitable or missed abortion.

They report as follows: "In the majority of cases the male frog pregnancy test differentiates in a short time between threatened abortion and inevitable, or missed, abortion. In some patients the test is negative almost immediately after the onset of uterine bleeding, but becomes positive again within twelve to twenty-four hours. Hence, in the absence of cervical effacement, dilatation, and excessive uterine bleeding, conservatism may be employed and the test repeated.

While waiting to repeat the test, any of the accepted forms of anti-abortive therapy may be carried out. If the second test is negative, only twenty-four hours, at the most, will have been lost by the patient. If the second test is positive, she has been given every possible chance to continue her pregnancy."

They reported the use of the male frog test on 16 patients with symptoms of threatened abortion. 6 of the cases were diagnosed as threatening abortions and due to therapeutic action the pregnancy was continued. The remaining 10 patients were diagnosed as inevitable or missed abortions and treated accordingly.

They further report: "One of the big advantages of the male frog pregnancy test is the rapidity with which the test becomes negative after the abortion becomes inevitable. With the Aschheim-Zondek or the Schneider-Friedman tests, the persistence of the positive reaction does not necessarily imply that the embryo is still living. The pregnancy reaction may persist 7–10 days after the termination of a pregnancy. In the male frog test it becomes negative within 10–12 hours."

"The threatened abortion rate is 16 per cent or higher. If it were possible to differentiate between the patients with inevitable abortions and those with threatened abortions, the attending physician could manage each patient more intelligently. The patients with inevitable or missed abortions, if diagnosed early, would be saved long periods of bed rest and valueless medication. Patients evidencing threatened abortions would receive proper treatment, and a pregnancy which might otherwise have been lost, either from lack of therapy or by the institution of active treatment to evacuate the uterus, might be salvaged."

One test performed in this laboratory appears to verify these observations. In this case, a test was performed simultaneously on the rabbit and the frog. The rabbit gave a positive result and the frog was negative. From these results, the patient was diagnosed as an inevitable abortion and the diagnosis was proven correct when the patient aborted five days later.

An attempt was made to verify these observations further by testing a series of women with a higher expected rate of abortion. Urines were tested from women undergoing therapeutic treatment for venereal disease at the Central Ohio Rapid Treatment Center in Columbus. However, no tests were encountered which appeared to have any bearing on these applications.

SUMMARY

1. In February, 1948, Wiltberger and Miller reported the use of the male *Rana pipiens* frog as a new test animal for the diagnosis of pregnancy.

2. The injection of urine from a pregnant woman induces the emission of spermatozoa in the urine of a male frog. The spermatozoa can easily be detected by microscopic examination without special histologic preparation. A result can usually be determined within 2 hours after injection.

3. The methods reported in this paper offer several slight modifications in the treatment of toxic urine and the technic of injection reported by other authors.

4. A total of 211 tests has been reported with an overall accuracy of 98.33% where the results could be confirmed. In 104 cases of known pregnancies results were accurate in 98.08%. Results of comparisons between the frog test and the Friedman test on suspected pregnancies were coincident in 96.67% of the tests compared. Tests performed on women after parturition reveal that the reaction usually becomes negative within 24 hours. In a series of control tests on non-pregnant women and men, only negative results were obtained. A false positive test has not been encountered.

5. It appears that chorionic gonadotropin secreted by the placenta is the activating agent which induces the reaction in the frog. This hormone varies in titer with the time of pregnancy and in individual cases. The male frog reacts to 30 international units of chorionic gonadotropin (Antuitrin "S") and is capable of indicating the hormone in the urine of pregnant women throughout all months of pregnancy.

6. Aside from routine pregnancy testing, this test also has proved valuable in the diagnosis of retained placental tissue and in the differentiation between threatened abortion and inevitable or missed abortion.

7. This test offers many advantages over previous tests in simplicity, speed of the reaction, clear end point, sensitivity, specificity, cost and abundance of test animals.

BIBLIOGRAPHY

1. Collip, J. B. Interrelationships Among Urinary, Pituitary and Placental Gonadotropic Factors. *Jn. Amer. Med. Assn.*, 1935, 104(7): 556–558.
2. Evans, H. M., Kohls, C. L. and Wonder, D. H. Gonadotropic Hormone in the Blood and Urine of Early Pregnancy. *Jn. Amer. Med. Assn.*, 1937, 108(4): 287–289.
3. Holmes, S. J. The Biology of the Frog. New York, The Macmillan Co., 1928.
4. Howell, W. H. Textbook of Physiology. Philadelphia, W. B. Saunders Co., 1947.
5. Kelly, G. L. and Woods, E. B. The Friedman Test for Pregnancy. *Jn. Amer. Med. Assn.*, 1937, 108(8): 615–617.
6. Mainini, C. G. Pregnancy Test Using the Male Toad. *Jn. Clin. Endocrin.*, 1947, 7(9): 653–658.
7. Mainini, C. G. Pregnancy Test Using the Male Batrachia. *Jn. Amer. Med. Assn.*, 1948, 138(2): 121–125.
8. Miller, D. F. and Wiltberger, P. B. Some Peculiarities of the Male Frog Test for Early Pregnancy. *Ohio Jn. Science*, 1948, XLVIII(3): 89–94.
9. Miller, D. F. Personal Communication.
10. Pickett, R. F., Wiltberger, P. B. and Miller, D. F. The Use of the Male Frog Test as an Aid in the Diagnosis of Retained Placental Tissue. *Ohio Jn. Science*, 1948, XLVIII(6): 246–247.
11. Robbins, S. L. Pregnancy Tests. *New Eng. Jn. Med.*, 1948, 239: 333–339; 369–373 (Aug. 26 and Sept. 2).

12. Robbins, S. L. and Parker, F. Jr. The Use of the Male North American Frog (*Rana pipiens*) in the Diagnosis of Pregnancy. *Endocrin.*, 1948, 42(4): 237–243.
13. Silbernagel, W. M., Patterson, J. B. and Pickett, R. F. The Significance of Bleeding in Early Pregnancy as Evidenced by the Male Frog Pregnancy Test. *Ohio Jn. Science*, 1948, XLVIII(6): 249–250.
14. Silbernagel, W. M. Personal Communication.
15. Turner, C. D. General Endocrinology. Philadelphia, W. B. Saunders Co., 1948.
16. Wiltberger, P. B. and Miller, D. F. The Male Frog, *Rana pipiens*, as a New Test Animal for Early Pregnancy. *Science*, 1948, 107(2773): 198.
17. Zondek, B., Sulman, F. and Black, R. Hormone Pregnancy Test. *Jn. Amer. Med. Assn.*, 1945, 128(13): 939–944.
18. Zondek, B. Gonadotropic Hormone in the Diagnosis of Chorionepithelioma. *Jn. Amer. Med. Assn.*, 1937, 108(8): 607–611.

THE DENISON SCIENTIFIC ASSOCIATION

REPORT OF THE SECRETARY

During the academic year 1947–8 the Association, under the direction of Professor R. H. Howe, President, carried out a program of meetings as outlined below. All of the lectures listed were presented on the basis of open invitation. The lecturers, unless otherwise indicated, were members of the faculty of Denison University.

September 23, 1947

PROTECTIVE MECHANISMS OF THE BODY AGAINST INJURY AND DISEASE. Dr. George D. Morgan

October 14, 1947

LIFE PROCESSES; A DISCUSSION. Professor W. Edward Deeds and Dr. A. W. Lindsey

October 28, 1947

ATOMIC BOMB TESTS AT BIKINI. (Illustrated). Dr. M. L. Pool, The Ohio State University

December 9, 1947

RESEARCH IN COMMERCIAL LIGHTING. Mr. Ruland L. Smith, The Holophane Company, Newark, Ohio

January 13, 1948

SYNTHETIC RUBBER. Professor Cecil R. Fetters

February 10, 1948

PREDICTION OF MUSICAL APTITUDE. Dr. Robert W. Lundin

March 9, 1948

MOVEMENT FOR FAMILY LIFE EDUCATION. Mrs. Esther F. Vreeland

March 23, 1948

GEOLOGY OF THE VERDE VALLEY. Professor Richard H. Mahard

April 13, 1948

MAGNETISM. Dr. Leon E. Smith

April 27, 1948

EMPIRE OF THE WEST. (With motion pictures) Professor A. Collins Ladner

May 11, 1948

Annual Dinner and Business Meeting, Caldwell Dining Hall

At the annual May meeting the following officers were elected to serve the Association for the year 1948–9.

President,	Dr. Arthur W. Lindsey
Vice-president,	Dr. Conrad E. Ronneberg
Secretary-Treasurer,	Dr. Marion Wetzel
Librarian,	Dr. Leon E. Smith
Secretary and Editor, *Journal of the Scientific Laboratories*,	Dr. W. Alfred Everhart

Note: Unforseen delays have caused the omission of the August 1948 issue of this *Journal*. In consequence the 1949 issues will be combined with those of 1948 to constitute Volume 41.

The Editor.

New Species of Bryozoans from the Pennsylvanian of Texas; Raymond C. Moore. 17 pp., 1 plate.

Articles 4–5, pp. 165–200, August, 1930.....$1.00
Visual Localization in the Horizontal Plane; Winford L. Sharp. 9 pp.
Petroleum Products for Internal Combustion Engines; Milton Finley. 26 pp., 3 figs.

Articles 6–7, pp. 201–299, December, 1930.....$1.00
The Actinoceroids of East-Central North America; Aug. F. Foerste and Curt Teichert. 96 pp., 33 plates.
The Presence of Nybyoceras in South Manchuria; Riuji Endo. 3 pp., 1 plate.

VOLUME 26

Article 1, pp. 1–142; April, 1931.....$1.50
The Hesperioidea of North America; A. W. Lindsey, E. L. Bell and R. C. Williams, Jr. 142 pp., 33 plates.

Article 2, pp. 143–250; December, 1931.....$1.50
The Older Appalachians of the South; Frank J. Wright. 108 pp., 38 plates.

VOLUME 27

Article 1, pp. 1–46; June, 1932.....$1.50
The Geomorphic Development of Central Ohio (Part I); Henry S. Sharp. 46 pp., 1 fig., 6 plates.

Article 2, pp. 47–136; December, 1932.....$1.50
Black River and Other Cephalopods from Minnesota, Wisconsin, Michigan, and Ontario (Part I); Aug. F. Foerste. 90 pp., 31 plates.

VOLUME 28

Articles 1–3, pp. 1–154; April, 1933.....$1.50
Black River and Other Cephalopods from Minnesota, Wisconsin, Michigan, and Ontario (Part II); Aug. F. Foerste. 146 pp.; for plates *cf.* This Journal, Vol. 27, Art. 2 (December, 1932).
A Study of the Change in Mass of the Anode of the Aluminum-Lead Cell; Charles E. Welling. 5 pp.
Balancing Chemical Equations—The Contribution of Otis Coe Johnson (1880); W. C. Ebaugh. 4 pp., 1 plate.

Article 4, pp. 155–248; October, 1933.....$1.00
The Ordos Desert of Inner Mongolia; George B. Cressey. 94 pp., 32 figs., 1 plate.

VOLUME 29

Article 1, pp. 1–105, April, 1934.....$1.25
The Newer Appalachians of the South (Part I); Frank J. Wright. 105 pp., 28 plates, 3 figs.

Article 2, pp. 107–193, August, 1934.....$1.25
Silurian Cyrtoconic Cephalopods from Ohio, Ontario, and Other Areas; Aug. F. Foerste. 86 pp., 14 plates.

Articles 3–4, pp. 195–238, December, 1934.....$1.00
Evidence of Ice Action in the Teays Valley, West Virginia; Julian J. Petty. 10 pp., 4 figs.
The Earlier History of the BULLETIN OF THE SCIENTIFIC LABORATORIES OF DENISON UNIVERSITY; Aug. F. Foerste. 23 pp.

VOLUME 30

Articles 1–2, pp. 1–118, April, 1935.....$1.00
Big Horn and Related Cephalopods; Aug. F. Foerste. 96 pp., 22 plates.
New Light Sources for Photographic Purposes; W. E. Forsythe. 22 pp., 7 figs.

Articles 3–4, pp. 119–230, August, 1935.....$1.00
Correlation of Silurian Formations in Southwestern Ohio, Southeastern Indiana, Kentucky and Western Tennessee; Aug. F. Foerste. 87 pp.
A Study of the Phenomenon of Wetting Films; C. W. Chamberlain and Kenneth Lyle Warren. 25 pp., 4 plates, 1 fig.

Articles 5–6, pp. 231–303, December, 1935.....$1.00
The Cephalopods of the Maquoketa Shale of Iowa; Aug. F. Foerste. 27 pp., 11 plates.
New Genera of Ozarkian and Canadian Cephalopods E. O. Ulrich and Aug. F. Foerste. 32 pp., 1 plate.

VOLUME 31

Articles 1–2, pp. 1–92, April, 1936.....$1.00
Hierosaurus colei; a new aquatic Dinosaur from the Niobrara Cretaceous of Kansas; M. G. Mehl. 20 pp., 3 plates.
Silurian Cephalopods of the Port Daniel Area on Gaspé Peninsula, in Eastern Canada; Aug. F. Foerste. 72 pp., 23 plates.

Article 3, pp. 93–142, August, 1936.....$1.00
The Newer Appalachians of the South (Part II): South of the New River; Frank J. Wright. 50 pp., 6 figs., 32 plates.

Articles 4–6, pp. 143–259, December, 1936.....$1.00
A Study of the Vestigial Air Bladder in the Darter (*Catonotis flabellaris rafinesque*); George David Morgan. 16 pp., 10 plates.
e Must Shape our New World (Commencement Address); Arthur Holly Compton. 11 pp.
Later History of the JOURNAL OF THE SCIENTIFIC LABORATORIES OF DENISON UNIVERSITY; Kirtley F. Mather. 25 pp.
Report of the Permanent Secretary of the DENISON SCIENTIFIC ASSOCIATION. 52 pp.

VOLUME 32

Articles 1–2, pp. 1–131, April, 1937.....$1.00
History of *Theta* Chapter of Ohio, Phi Beta Kappa (1911–1936); Willis A. Chamberlin. 69 pp., 2 figs.
The Tungsten Filament Incandescent Lamp; W. E. Forsythe and E. Q. Adams. 72 pp., 10 figs.

Articles 3–7, pp. 133–207, August, 1937.....$1.00
Science and the College (Commemoration Address); Carey Croneis. 12 pp.
Our Endowment (Commemoration Address); C. Judson Herrick. 9 pp.
Science—Man's Liberator (Commemoration Address); William E. Wickenden. 10 pp.
The Application of Physics to Modern Hydrographic Surveying; (Commemoration Address); Herbert Grove Dorsey. 22 pp., 10 plates.
An Annoted Check-list of Birds Recorded at Granville, Licking County, Ohio; Ward M. Klepfer and William J. Taylor. 21 pp., 1 plate.

Article 8, pp. 209–337, December, 1937.....$1.00
Upper Carboniferous Crinoids from the Morrow Subseries of Arkansas, Oklahoma and Texas; Raymond C. Moore and Frederick N. Plummer. 106 pp., 37 figs., 5 plates.
Report of the Permanent Secretary of the DENISON SCIENTIFIC ASSOCIATION. 10 pp.

VOLUME 33

Article 1, pp. 1–60, April, 1938.....$1.00
The Telescopic Alidade and the Plane Table as used in Topographic and Geologic Surveys; Kirtley F. Mather and Bradford Washburn. 60 pp., 19 figs.

Articles 2–3, pp. 61–155, August, 1938.....$1.00
Chemistry and the Modern Meat Packing Industry; Minard Patrick. 48 pp., 3 figs.
Certain Nuclear Masses in the Macaque Medulla Oblongata—Preliminary Report; Frederick David Goudie. 47 pp., 8 figs.

Articles 4–7, pp. 157–369, December, 1938.....$1.50
The Use of Fragmentary Crinoidal Remains in Stratigraphic Paleontology; Raymond C. Moore. 86 pp., 14 figs., 4 plates.
New Ostracodes from the Golconda Formation; Carey Croneis and Arthur S. Gale, Jr. 46 pp., 2 plates.
New Ostracodes from the Kinkaid Formation; Carey Croneis and Franklin A. Thurman. 34 pp., 2 plates.
New Ostracodes from the Clore Formation, Carey Croneis and Harold J. Funkhouser. 29 pp., 2 plates.
Report of the Permanent Secretary of the DENISON SCIENTIFIC ASSOCIATION. 8 pp.

VOLUME 34

Articles 1–3, pp. 1–63, April, 1939.....$1.00
Some Factors Affecting the Operation of Incandescent Lamps; W. E. Forsythe, E. Q. Adams, and P. D. Cargill. 27 pp., 7 figs.
Taxonomy of Chester Ostracodes; Carey Croneis. 4 pp.
New Ostracodes from the Renault Formation; Carey Croneis and Ralph L. Gutke. 31 pp., 2 plates.

Articles 4–5, pp. 65–169, August, 1939.....$1.00
New Ostracodes from the Menard Formation; Carey Croneis and Hubert M. Bristol. 38 pp., 2 plates.
Pioneering in Yeneseiland. A transect across Siberia from Mongolia to the Arctic; George B. Cressey. 67 pp., 51 figs.

Article 6, pp. 171–294, December, 1939.....$1.00
New Crinoids from Upper Pennsylvanian and Lower Permian Rocks of Oklahoma, Kansas, and Nebraska; Raymond C. Moore. 109 pp., 39 figs., 5 plates.
Report of the Permanent Secretary of the DENISON SCIENTIFIC ASSOCIATION. 8 pp.

VOLUME 35

Articles 1–2, pp. 1–54, April, 1940.....$1.00
The Relative Efficiency of Single Versus Multiple Ex-

posures in the Rapid Memorization of Visual Forms; L. C. Steckle. 31 pp., 7 figs.
New Genera of Pennsylvanian Crinoids from Kansas, Oklahoma and Texas; Raymond C. Moore. 23 pp., 9 figs., 1 plate.

Article 3, pp. 55-137, August, 1940 ..... $1.00
Relationships of the Family Allagecrinidae, with Description of New Species from Pennsylvanian Rocks of Oklahoma and Missouri; Raymond C. Moore. 83 pp., 14 figs., 2 plates.

Articles 4-8, pp. 139-218, December, 1940 ..... $1.00
Gas Amplification of Photo-electric Currents for High Values of *E/P*; Neil Edward Handel. 28 pp., 15 figs., 1 plate.
Caney Conodonts of Upper Mississippian Age; E. B. Branson and M. G. Mehl. 12 pp., 1 plate.
Conodonts from the Keokuk Formation; E. B. Branson and M. G. Mehl. 10 pp., 1 plate.
A Record of Typical American Conodont Genera in Various Parts of Europe; E. B. Branson and M. G. Mehl. 6 pp., 1 plate.
The Recognition and Interpretation of Mixed Conodont Faunas; E. B. Branson and M. G. Mehl. 15 pp.
Report of the Permanent Secretary of the DENISON SCIENTIFIC ASSOCIATION. 8 pp.

VOLUME 36

Articles 1-3, pp. 1-66, April, 1941 ..... $1.00
Tegminal Structure of the Pennsylvanian-Permian Crinoid *Delocrinus*; Raymond C. Moore and Harrell L. Strimple. 12 pp., 1 plate.
Fluorescence and Fluorescent Lamps; W. E. Forsythe, B. T. Barnes and E. Q. Adams. 34 pp., 17 figs.
Dakotasuchus Kingi, A Crocodile from the Dakota of Kansas; M. G. Mehl. 19 pp., 3 figs., 2 plates.

Articles 4-5, pp. 67-133, August, 1941 ..... $1.00
What Students Want in an Instructor; L. C. Steckle. 4 pp.
Chemistry and Modern Laundry Practice; Earl R. Haynes. 63 pp., 3 figs.

Articles 6-8, pp. 135-174, December, 1941 ..... $1.00
Illinoian Glaciation in Killbuck Valley South of Millersburg, Ohio; George D. Hubbard. 11 pp., 1 fig.
Biological Sociology; Alfred Edwards Emerson. 10 pp.
The Life Science Building at Denison University; Arthur Ward Lindsey. 4 pp., 1 fig.
Report of the Permanent Secretary of the DENISON SCIENTIFIC ASSOCIATION. 15 pp.

VOLUME 37

Articles 1-2, pp. 1-66, April, 1942 ..... $1.00
A Preliminary Revision of Hesperia; Arthur Ward Lindsey. 50 pp., 6 plates.
The Growth of the Tourist Court in the United States, and its Relationship to the Urban Development of Albuquerque, New Mexico; Franklin T. McCann. 11 pp., 5 plates.

Articles 3-7, pp. 67-132, August, 1942 ..... $1.00
Megaliocrinus, A new Camerate Crinoid Genus from the Morrow Series of Northeastern Oklahoma; Raymond C. Moore and Lowell R. Laudon. 10 pp., 5 figs.
Metacatillocrinus, A new Inadunate Crinoid Genus from Pennsylvanian Rocks of Oklahoma; Raymond C. Moore and Harrell L. Strimple; 8 pp., 6 figs.
Blastoids from Middle Pennsylvanian Rocks of Oklahoma; Raymond C. Moore and Harrell L. Strimple; 7 pp., 1 fig.
A New Species of Synbathocrinus from Mississippian Rocks of Texas, with Description of Ontogeny; Raymond C. Moore and John D. Ewers. 15 pp., 28 figs.
Mercury Vapor Lamps; W. E. Forsythe, E. Q. Adams and B. T. Barnes. 26 pp., 20 figs.

Articles 8-9, pp. 133-163, December, 1942 ..... $1.00
Geography in a World at War; C. Langdon White. 7 p.
The Meaning of Science in Human Affairs; C Judson Herrick. 13 pp.
The Denison University Research Foundation. 1 p.
Report of the Permanent Secretary of the DENISON SCIENTIFIC ASSOCIATION. 7 pp.

VOLUME 38

Articles 1-2, pp. 1-39, April, 1943 ..... $0.75
Establishing and Maintaining a Color Temperature Scale; W. E. Forsythe and E. Q. Adams. 31 pp., 9 figs.
The Vegetation of Idaho; R. Maurice Myers. 8 pp., 1 fig.

Articles 3-4, pp. 41-76, December, 1943 ..... $0.75
The Cranial Nerves. A Review of Fifty Years; C Judson Herrick. 12 pp.
Radiometric and Colorimetric Characteristics of the Blackbody between 2800°K and 3800°K; E. Q. Adams and W. E. Forsythe. 17 pp., 1 fig.
Report of the Permanent Secretary of the DENISON SCIENTIFIC ASSOCIATION. 8 pp.

VOLUME 39

Article 1, pp. 1-54, June, 1945 ..... $0.75
The Disappearing-filament Optical Pyrometer Calibration and Use; W. E. Forsythe and E. Q. Adams. 48 pp. and 18 figs.
Report of the Permanent Secretary of the DENISON SCIENTIFIC ASSOCIATION for 1944. 5 pp.

Article 2, pp 55-90, December, 1945 ..... $0.75
Photometry; Field Brightness and Eye Adaptation; W. E. Forsythe and E. O. Adams. 29 pp. and 4 figs.
Report of the Permanent Secretary of the DENISON SCIENTIFIC ASSOCIATION for June to December, 1945. 7 pp.

Articles 3-7, pp. 91-170, June, 1946 ..... $1.00
Dyadic Operator Approach to a Study in Conics, with Some Extension to Higher Dimensions; James Loyd Shawn. 15 pp.
Dyadic Operator as Applied to Lines, Planes and Quadric Surfaces; Laura Lee Cowen. 7 pp.
Spectrograph and its Use in Spectrochemical Analysis; Betty Jane Oestmann. 21 pp., 5 figs.
Synthetic Rubbers; William Farrand Loranger and Robert Garfield Anderson. 22 pp., 2 figs.
Role of Suggestibility in Susceptability to the Size-Weight Illusion and the Phenomenon of Autokinetic Streaming; Suzanne Littell. 75 pp., 2 figs.

Articles 8-9, pp. 171-194, December, 1946 ..... $1.00
Existence and Condition of Minimum Deviation of a Prism; Robert E. Seall. 4 pp., 1 fig.
Natural History Collections of Denison University; C. Judson Herrick. 8 pp.
Report of the Permanent Secretary of the DENISON SCIENTIFIC ASSOCIATION, 12 pp.
Subject and Author Index, January, 1944 to December, 1946. 5 pp.

VOLUME 40

Articles 1-3, pp. 1-32, April, 1947 ..... $1.00
In Appreciation. A Report by the Editor; 2 pp., 1 plate
Conodonts from the Fern Glen of Missouri; M. G. Mehl and Leo A. Thomas. 18 pp., 1 plate
Where do we Stand with Cancer? Ralph E. Pickett. 11 pp.

Article 4, pp. 33-72, August, 1947 ..... $1.00
Metric Differential Geometry of Reciprocal Retilinear Congruences; Winthrop W. Dolan. 34 pp.
Program of the Denison Scientific Association. A Report by the Permanent Secretary; 5 pp.

Articles 5-6, pp, 73-115, December, 1947 ..... $1.50
The Autokinetic Test as a Measure of Introversion-Extroversion; Marcia Helen Voth. 14 pp.
A Comparative Study of the Variations of the Postrenal Vena Cava of the Cat and the Rat and a Description of Two New Variations; Norman B. Abell. 28 pp.; 23 plates.

VOLUME 41

Article 1, pp. 1-38; April, 1948 ..... $1.00
The Role of Security-Insecurity Feelings in Liberal and Conservative Attitudes; Martha F. Sturm. 38 pp., including 10 figs., 13 tables.

Articles 2-3, pp. 39-66; December, 1948 ..... $1.25
Observations on the Michigan Flora: A Survey of the St. Ignace Causeway (Mackinac County); Edward G. Voss. 7 pp.; 2 plates.
The Male Frog Pregnancy Test; Carl F. Brandfass, Jr. and Emil J. Massa; 20 pp.; 3 plates.
Report of the Secretary; THE DENISON SCIENTIFIC ASSOCIATION; 1 p.